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# Graded response to short photoperiod during development and early adulthood in Siberian hamsters and the effects on reproduction as females age

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ABSTRACT

Short day (SD) lengths delay puberty, suppress ovulation, inhibit sexual behavior, and decelerate reproductive aging in female Siberian hamsters (*Phodopus sungorus*). To date, the modulation of the age-associated decline in reproductive outcomes has only been demonstrated in female hamsters experiencing different day lengths during development. To determine if developmental delay is necessary for photo-inhibition to decelerate reproductive aging, hamsters raised in LD were transferred to SD as young adults and remained there for 6 months. Females that demonstrated the most immediate and sustained photo-inhibition were found to have greater numbers of ovarian primordial follicles at advanced ages (9 and 12 months) than did females held in LD, nonresponders to SD, and females with a marginal SD-response. Similarly, for females raised in SD from conception to 6 months of age, prolonged developmental delay was associated with greater numbers of primordial follicles at later ages as compared to hamsters that became refractory to SD. A robust response to SD in juvenile and adult hamsters is associated with decelerated reproductive aging, which may result in greater reproductive success in older females as compared to age-matched individuals demonstrating a more modest response to SD.



## Introduction

The life history strategy of seasonally breeding rodents is strongly influenced by the photoperiodic conditions at the time of birth and during development. Young born before or near the summer solstice mature quickly, and females are likely to produce offspring in the year of their birth (Bronson, 1985). Conversely, when the breeding season extends past the summer solstice into late summer and early autumn, as day length decreases, young individuals are more likely to delay puberty and defer their first reproductive effort until the following spring (Butler et al., 2007).

In Siberian hamsters, *Phodopus sungorus*, raising females in short days (SD) was associated with a delay in reproductive aging (Place et al., 2004). Decelerated reproductive aging in SD females, as compared to females held in long days (LD), was manifested as greater reproductive success when LD and SD females were first paired with males at 9 months of age. Additionally, SD females had significantly more ovarian primordial follicles than LD hamsters at 3 and 6 months of age (Place et al. 2004), and this advantage persisted through 12 months of age, even though SD females had been transferred to a long photoperiod 4 months prior (unpublished results). Primordial follicles represent the resting pool of germ cells in the mammalian ovary, and their numbers decline with age because activation of follicular growth is irreversible and occurs throughout a female's lifetime (Zuckerman, 1951; vom Saal et al., 1994). In women, when ovarian follicles are completely depleted, or nearly so, the menopause ensues.

Because puberty is significantly delayed in Siberian hamsters raised in SD, the deceleration of reproductive aging may reflect a shift in their life history trajectory that results from delayed maturation. However, hamsters transferred to SD as adults also demonstrate a profound inhibition of reproductive physiology — gonadotropin levels are suppressed relative to LD hamsters (Dodge and Badura, 2002; Kenny et al., 2002), ovulation ceases, the uterus decreases in size, and the vaginal opening closes (Lerchl and Schlatt, 1993; Schlatt et al., 1993). To determine if SD after puberty affects reproductive aging, we compared outcomes in older female hamsters held continuously in LD to that of females exposed to 6 months of SD either before or after puberty. If the attrition of primordial follicles is not tempered by SD exposure after puberty, then pubertal delay assumes greater importance in the previously described account of SD-induced deceleration of reproductive aging (Place et al., 2004). This finding would also suggest that females in the wild that mature during the year of their birth, may experience continued reproductive decline even if they respond to decreasing day length and suppress their reproductive physiology. As such, if they survive to the next spring's breeding season their capacity to breed successfully may be reduced, especially compared to overwintering females that delayed puberty (Schwarz et al., 1964).

In addition to counting ovarian follicles, serum concentrations of anti-Müllerian hormone (AMH) were also measured because this

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hormone has been found to reflect the size of follicular pool in mice (Kevenaar et al., 2006) and in women (van Rooij et al., 2005). AMH is produced by ovarian granulosa cells of growing follicles (Baarends et al., 1995), but we have also noted its expression in hypertrophied granulosa cells that surround atretic oocytes in ovaries from Siberian hamsters raised in SD (Kabithe and Place, 2008). These hypertrophied granulosa cells have a luteinized histological appearance, and they occupy a large majority of the ovarian volume in SD hamsters, which appears to account for the three-fold higher levels of AMH in SD as compared to LD hamster ovaries (Kabithe and Place, 2008). Moreover, AMH inhibits primordial follicle activation in mice (Durlinger et al. 1999, 2002), and thus higher AMH in the ovaries of hamsters held in SD may manifest as greater numbers of primordial follicles.

The physiological responses to short or decreasing photoperiod go beyond inhibition of the reproductive axis, as Siberian hamsters in SD manifest differences in food intake, body mass, and pelage (Lerchl and Schlatt, 1993, Knopper and Boily, 2000). These reactions to changing environmental conditions are thought to increase the chances of overwinter survival as resources diminish and energetic demands of foraging and temperature regulation increase (Dark and Zucker, 1985). Similarly, modulation of the immune system by photoperiod has been observed in Siberian hamsters (Bilbo et al., 2002; Prendergast et al., 2004; Demas and Sakaria 2005; Weil et al., 2006), which has been postulated to be adaptive in the context of seasonally variable immune challenges and energetic demands (Prendergast et al., 2001). The T-cell mediated antibody response to a naïve antigen (keyhole limpet hemocyanin, KLH) has been reported to be lower in SD than in LD hamsters (Yellon et al., 1999; Drazen et al., 2001; Demas, 2002), but to date all studies have evaluated relatively young adults. In the present study we assessed the antibody response to KLH challenge in hamsters ranging from 3 to 12 months of age, to determine if the response deteriorates with age, and if so, is that age-associated decline modulated by photoperiodic history. We anticipated a lower KLH-antibody response in the oldest LD animals, because of the reported changes in T-cell subsets with age in mice (Miller, 1996), with a shift from naïve to a larger proportion of memory T-cells. Seeing as reproductive and somatic aging are so thoroughly intertwined (Williams, 1966; Stearns, 1992), we set out to determine if a photoperiodic history that decelerates reproductive aging will also modulate a potential biomarker of somatic aging.

## Methods and materials

### *Experimental animals*

Siberian hamsters from our colony (14 h of light per day, 14 L) were transferred to LD (16 L) or SD (10 L) as breeding pairs to generate females for the following experiment. Experimental females were assigned to one of three groups (Table 1). The time of lights-off was synchronized for all animals to 1700 Eastern Standard Time (EST). Animals were originally derived from wild-bred stock obtained from Dr. K. Wynne-Edwards, Queen's University. Hamsters were weaned on postnatal day 18, ear-tagged for identification, weighed, and placed in polypropylene cages (2 to 4 siblings/cage). Food (Teklad 8626, Madison, WI) and water were available ad libitum. Ambient temperature and relative humidity were held constant between 21 °C ± 5 and 50 ± 10%, respectively. Body mass, coat color, and vaginal patency were assessed and recorded weekly. Experimental procedures were approved by Cornell University's Institutional Animal Care and Use Committee and conducted in accordance with the NRC Guide for the Care and Use of Laboratory Animals.

Five to eight animals from each group were killed at 3, 6, 9 and 12 months of age to collect blood and harvest reproductive tissues (details below). Females failing to respond to SD (maintained open vagina, summer pelage, and body mass) were excluded from the 3, 6, and 9-month-old cohorts. However, a sufficient number of photo-nonresponders (NR) from within the LD–SD–LD group were available at the 12-month sampling point to include them as a fourth group. The essential study design was meant to determine if 6 months in SD results in a greater preservation of ovarian primordial follicles as compared to females held in LD, independent of the timing of the SD exposure relative to puberty.

**Table 1**
Group designations for experimental females

| Groups | Day lengths during 3-month age intervals | | | |
|---|---|---|---|---|
| | 0–3 mo | 3–6 mo | 6–9 mo | 9–12 mo |
| LD | 16 L | 16 L | 16 L | 16 L |
| LD–SD–LD | 16 L[a] | 10 L | 10 L | 16 L |
| SD–LD | 10 L | 10 L | 16 L | 16 L |

LD-hamsters were held in 16 L throughout. LD–SD–LD-females were raised in 16 L and transferred to 10 L at 3 months of age, where they remained until 9 months of age, when they were transferred back to 16 L. SD–LD-females were raised in 10 L and transferred to 16 L at 6 months of age. Blood and tissues were collected from six animals from each group at 3, 6, 9, and 12 months of age. Gray shading indicates times when the LD–SD–LD- and SD–LD-females were held in SD.

[a] The photoperiodic histories of LD- and LD–SD–LD-females were identical through 3 months of age, thus in actuality there were only two groups at this sampling age.

### *Blood and tissue collection*

Females from each group were killed at predetermined ages (3, 6, 9 and 12 mo) by intraperitoneal overdose of sodium pentobarbital and exsanguination by retro-orbital bleed. All animals were euthanized during the middle of the light cycle. Blood was clotted on ice at room temperature for 1 h and centrifuged at 3600 rpm for 20 min in 4 °C. Drawn off serum was aliquoted, frozen, and maintained at −80 °C until assayed for AMH and KLH-antibodies.

The right ovary was removed from each animal, dissected free of surrounding fat, weighed on an analytical balance, and immersed in 10% buffered formalin for histology and follicle counts. The left ovary was removed, flash-frozen on dry ice, and stored at −80 °C for another study. The uterus was then removed, dissected free of surrounding fat, and weighed on an analytical balance. Formalin fixation of the right ovary continued overnight at room temperature, followed by serial dehydration into 70% ethanol. Ovaries were embedded in paraffin and serially sectioned at 6 μm.

### *Quantification of ovarian follicles*

Every tenth section was stained with hematoxylin and eosin (H&E; 3- and 6-month-olds) or Periodic-acid Schiff (PAS; 9- and 12-month-olds) and viewed under 400× magnification to count ovarian follicles. Because PAS staining allows for better visualization of the zona pellucida, our lab transitioned to this staining technique between the 6- and 9-month-old cohorts. A sampling of adjacent ovarian sections from 9-month-old females were stained with H&E or PAS and yielded comparable follicle counts. However, counting follicles required less time when sections were stained with PAS than with H&E, thus PAS was the method used for the older cohorts. Ovarian sections of poor histological quality were replaced with the adjacent section. For some ovaries, especially from the 6-month-old cohort, good quality replacement sections could not be found, resulting in their exclusion from the follicle count data.

Ovarian follicles were classified as primordial, transitional, primary, secondary, or antral. Primordial follicles were defined as an ooctye surrounded by a single layer of flattened granulosa cells, whereas transitional follicles contained a mix of flattened and cuboidal granulosa cells. Meredith et al., (2000) referred to transitional follicles as type B/C follicles and considered them to be part of the pool of primordial follicles, because this class of follicle consists of both slowly growing and non-growing follicles. Therefore, primordial and transitional follicles counts were combined and presented as primordial follicle counts. Primary follicles had a single layer of